RANKL/RANK/OPG: new therapeutic targets in bone tumours and associated osteolysis.

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Acknowledgements

This work was supported by a CReS INSERM n° 4CR06F, by a grant from the French Ministry of Research and Technology (TS/02 2 0044) and by a grant from the Loire-Atlantique Committee of the Ligue Contre le Cancer. S. Théoleyre and Y. Wittrant received fellowships, respectively from the Loire-Atlantique Committee of the Ligue Contre le Cancer and from the Région des Pays de Loire.

Abstract

The emergence of the molecular triad osteoprotegerin (OPG)/Receptor Activator of NF-kB (RANK)/RANK Ligand (RANKL) has helped elucidate a key signalling pathway between stromal cells and osteoclasts. The interaction between RANK and RANKL plays a critical role in promoting osteoclast differentiation and activation leading to bone resorption. OPG is a soluble decoy receptor for RANKL that blocks osteoclast formation by inhibiting RANKL binding to RANK. The OPG/RANK/RANKL system has been shown to be abnormally regulated in several malignant osteolytic pathologies such as multiple myeloma, where enhanced RANKL expression (directly by tumour cells or indirectly by stromal bone cells or T-lymphocytes) plays an important role in associated bone destruction. By contrast, production of its endogenous counteracting decoy receptor OPG is either inhibited or too low to compensate for the increase in RANKL production. Therefore, targeting the OPG/RANK/RANKL axis may offer a novel therapeutic approach to malignant osteolytic pathologies. In animal models, OPG or soluble RANK were shown both to control hypercalcaemia of malignancy and the establishment and progression of osteolytic metastases caused by various malignant tumours. To this day, only one phase I study has been performed using a recombinant OPG construct that suppressed bone resorption in patients with multiple myeloma or breast carcinoma with radiologically confirmed bone lesions. RANK-Fc also exhibits promising therapeutic effects, as revealed in animal models of prostate cancer and multiple myeloma. If the animal results translate to similar clinical benefits in humans, using RANK-Fc or OPG may yield novel and potent strategies for treating patients with established or imminent malignant bone diseases and where standard therapeutic regimens have failed.

Introduction

The invasion of bone tissue by a benign or malignant tumour, primary or secondary, rapidly affects the balance between bone resorption and apposition. In some rare cases, tumour development leads to osteoformation without osteolysis, as in some forms of osteosarcoma or osteoblastic metastasis predominating in patients with prostatic adenocarcinoma. In most cases, the skeletal manifestation of malignancy is focal osteolysis. This imbalance in favour of bone resorption can result from the acquisition of new cellular properties by bone cells : increase in the proteolytic activity, alteration in local or humoral factors expression. One hypothesis is that genomic instability of tumour cells (bone metastases, bone sarcoma, giant cell tumours) might cause mutations that affect critical cellular properties. Another hypothesis is the building of an environment favouring bone resorption (essential cyst)[1]. Whatever the causes and the mechanisms, tumour-induced osteolysis is responsible for high morbidity. Severe bone pain remains the first symptom, as revealed in the majority of benign tumours and in 60 to 70% of bone metastases, pathological fractures, nerve compression syndromes (paralysis) and profound hypercalcaemia (in secondary tumours only). Osteolysis must occur before tumour cells can grow and invade the mineralised bone. Although *in vitro* studies demonstrated that breast cancer cells can directly resorb bone [2], the majority of evidence shows that the primary mechanism responsible for bone destruction in patients with cancer is tumour-mediated stimulation of osteoclastic bone resorption. Osteoclasts appear to be the primary bone-resorbing cells both in normal and pathological states. Increased osteoclastic bone resorption results from both increased osteoclast generation and induction of pre-existing osteoclast to resorb bone. Tumour products can either stimulate osteoclast formation locally in the bone microenvironment or systemically through production of hormones such as parathyroid hormone-related protein (PTH-rP), the mediator of the humoral hypercalcaemia of malignancy. Other factors produced by tumour cells that can stimulate osteoclastic bone resorption include interleukin-1 (IL-1), IL-6, tumour necrosis factor-alpha (TNF-α) and macrophage inflammatory protein-1-alpha (MIP-1α)[3]. These agents, released into the bone microenvironment, act on osteoblastic stromal cells to enhance the production of osteoclast activating factors. Most notable of these is Receptor Activator of NF-kB Ligand (RANKL), which is a recent addition to the TNF gene family. Ample data from the literature highlights the critical role of RANKL in mediating tumour-induced bone destruction, in both primary and secondary bone tumours. Therefore, the development of therapeutic agents disrupting the

interactions of RANKL and its cognate receptor RANK represents promising new options for the treatment of patients with primary and secondary bone tumours.

I. Involvement of RANKL/OPG in malignant bone diseases

RANKL is a potent osteoclastogenic factor that, in combination with macrophage colony-stimulating factor (M-CSF), induces osteoclast formation *in vitro*. RANKL is expressed as a membrane-bound protein on the surface of osteoblasts, osteocytes and marrow stromal cells [4]. In addition, activated T cells secrete RANKL as a soluble molecule [5]. Furthermore, most osteotropic factors such as IL-1, IL-11, prostaglandin E_2 and 1,25-$(OH)_2D_3$ induce osteoclast formation by binding to marrow stromal cells, which in turn express increased levels of soluble or membrane forms of RANKL. RANKL then binds to its receptor RANK, present at the surface of osteoclast precursors and mature osteoclasts, inducing osteoclast formation and activation [6]. RANKL activity can be blocked by the soluble decoy receptor osteoprotegerin (OPG), resulting in prevention of bone resorption [7]. OPG, a recently described member of the TNF receptor superfamily, is produced by a lot of cell types, such as bone-marrow stromal cells and osteoblasts, and blocks the fusion/differentiation stage of osteoclast precursors, rather than the proliferation stage, by binding to RANKL. Thus, the RANKL/RANK/OPG system produced by the bone microenvironment represents important and final cytokines of osteoclast biology, the ratio RANKL to OPG regulating in fine the orientation to bone formation or bone resorption. In the case of tumour-associated osteolysis, several studies have implicated the cytokines RANKL and OPG as essential regulators of tumour-bone interactions (osteolytic bone metastases, humoral hypercalcaemia of malignancy) [8].

Modulation of RANKL and OPG expression has been reported in several bone tumours where osteolysis occurs (Table 1). In a prospective immunohistochemical study, Good *et al.* demonstrated that primary benign and malignant bone tumours and metastasis were positive for RANKL (13 cases of 16)[9]. More recently, Grimaud *et al.* (2003) demonstrated by RT-PCR and ELISA that the RANKL/OPG ratio was significantly increased in patients suffering from severe tumour-associated osteolysis compared to healthy tissues [10]. Moreover in the same study, RANKL expression was demonstrated immunohistochemically in primary malignant bone tumours (osteosarcoma and chondrosarcoma) and in Giant Cell Tumor (Figure 1). OPG was shown to co-localize with RANKL, suggesting that OPG expression may reflect a homeostatic mechanism of the skeleton to counterbalance the increased bone resorption.

- *Myeloma*

The OPG/RANKL system has been widely studied in multiple myeloma-induced osteolysis. Multiple myeloma (MM) is a plasma cell malignancy that develops in the bone marrow and characterized by the development of osteolytic bone lesions associated with bone pain, hypercalcemia and pathological fractures. Three cell types are involved in this pathology : myeloma cells, bone marrow stromal cells and osteoclasts. Several data from the literature have reported that myeloma cells are able to induce an imbalance in the OPG/RANKL system in bone environment, and that this is responsible for osteolysis observed in patients. Immunohistochemical staining of bone marrow biopsies from patients with MM shows an increase of RANKL and a reduction of OPG expression [11]. Roux *et al.* reported that RANKL is over-expressed in stromal cells at the interface of MM with normal bone marrow elements [12]. However, there is controversy as to whether myeloma cells directly express RANKL [11-17]. In addition to increasing RANKL expression, MM-infiltrated bone marrow exhibits decreased production of the natural RANKL inhibitor OPG. Serum OPG levels were shown to be reduced in patients with multiple myeloma with lytic bone disease [18,19]. Two mechanisms are involved in this process. First, cell-to-cell contacts between myeloma cells, bone marrow stromal cells and osteoblasts inhibit OPG expression and production by stromal cells, as evidenced by co-culture experiments [11,16]. Second, syndecan-1, a transmembrane proteoglycan that is highly expressed at the surface of myeloma cells, binds and sequesters OPG through interaction with the heparin-binding domain of the OPG protein [20]. OPG is thereby internalised and degraded within the lysosomal compartment of myeloma cells. Thus, inhibition of OPG production by myeloma cells, both at transcriptional and post-translational levels, is associated with increased expression of RANKL in MM and disrupts the RANKL/OPG ratio in favour of the osteoclastogenesis factor RANKL. In a recent study, Terpos *et al.* demonstrate that the RANKL/OPG ratio is increased in MM and correlates with markers of bone resorption, osteolytic lesions and markers of disease activity, thus confirming for the first time in humans the importance of RANKL/OPG in the development of bone disease [21]. The authors therefore propose soluble RANKL/OPG ratio as a predictive survival index in multiple myeloma. In another hematologic malignancy, adult T-cell leukaemia cells from patients with hypercalcaemia strongly expressed the transcripts of the RANKL gene and induced *in vitro* the differentiation of human hematopoietic precursor cells into osteoclasts in the presence of M-CSF [22]. Direct contact between adult T-leukaemia cells and hematopoietic precursor cells was essential for the differentiation, suggesting that T-

leukaemia cells induce the differentiation of hematopoietic precursor cells to osteoclasts through RANKL expressed on their surface.

- *Skeletal metastases*

RANKL involvement is also demonstrated in the case of skeletal metastases secondary to breast and prostate carcinoma. Indeed, the development of osteolytic bone metastases and humoral hypercalcaemia of malignancy are frequent clinical complications of breast and prostate carcinoma, and both disorders involve an excessive activation of osteoclast via the RANKL-RANK interaction. Huang *et al* have investigated the expression of RANKL by *in situ* hybridisation and immunohistochemistry in the skeletal lesions of 16 patients with carcinomas that had metastasised to the bone [23]. Both RANKL mRNA and protein were present in more than 90% of metastatic tumour cells in adenocarcinoma lesions (from breast, prostate, lung or thyroid origin). With regard to the measurement of soluble RANKL, few studies have measured this ligand in the circulation of patients with malignant disease [24], although there is abundant evidence that RANKL mRNA is expressed in cells derived from the breast cancer stroma [25]. Results from this study and others suggest that the RANKL/OPG ratio is variable depending on the phenotype of the prostate cancer cells [26,27]. Other studies have reported that prostate cancer cells were shown to produce both OPG and RANKL, enabling then to influence bone metabolism through inhibition (OPG) and stimulation (RANKL) of osteoclastogenesis [28-30]. Immunohistochemical studies suggest that both OPG and RANKL are present *in vivo* in prostatic cancers, with higher levels of OPG detected in bone metastases than in primary tumours and metastases from non-osseous sites [29]. Few studies have determined OPG levels in patients with metastatic disease to the bone. In a recent study from Lipton *et al*, the serum OPG levels were measured in patients with solid tumours and metastatic diseases [19]. The results showed that, although some patients have significant levels of circulating OPG, these concentrations do not approach the level that would be expected to counterbalance the elevated RANKL production, thus suppressing osteoclast function. Jung *et al.* examined the serum level of OPG, in relation with the tumor dissemination, in patients with prostate cancer [31]. These authors showed that increased serum OPG is a marker of bone metastatic spread in prostate cancer patients. The same team recently studied the behaviour and diagnostic usefulness of RANKL and OPG in the serum of patients with prostate cancer [32]. While OPG was increased in patients with bone metastases, as previously demonstrated, RANKL levels did not differ among the control and prostate cancer groups. In this case, serum OPG but not RANKL indicates disrupted

osteoclastogenesis in patients with prostate cancer and bone metastatic spread and could be used as a marker for bone metastases. Thus, the OPG/RANKL ratio showed behaviour similar to that of OPG.

OPG further affects cancer cells, suppressing TRAIL (Tumor necrosis factor-related apoptosis-inducing ligand)-induced tumour cell apoptosis by binding to TRAIL. This was demonstrated with conditioned medium from co-cultures of MG63 cells with myeloma cells, which had a reduced effect on TRAIL-induced apoptosis, reflecting the decreased concentrations of OPG in co-cultures of myeloma cells with bone cells [33]. From these experiments, OPG may be considered as a survival factor for myeloma cells. The same results and conclusions were reported in Holen's study using prostate cancer cells : a strong negative correlation was observed between levels of endogenously produced OPG in the medium and the capacity of TRAIL to induce apoptosis in cells that produced high levels of OPG [30]. In relation with this activity, it has been hypothesized that the ability to produce OPG by cancer cells would confer them a survival advantage.

- *Giant Cell Tumour of bone*

RANKL/OPG involvement in bone tumours has also been demonstrated in Giant Cell Tumour (GCT) of bone, a rare primary osteolytic tumour characterized by massive bone destruction at the epiphysis of long bones. Histologically, it is characterized by a large number of multinucleated giant cells, macrophage-like and stromal-like mononuclear cells. There is no evidence that tumour cells themselves are capable of bone destruction. It appears instead that the tumour cells of GCT act by promoting osteoclastogenesis. However, the expression of OPG, RANK and RANKL seems to be cell type-dependent in GCT [34,35]. The ratio of RANKL and OPG gene expression in tumour cells may thus determine local osteoclastogenesis. Concurrently, the study of Miyamoto *et al.* confirm that spindle-shaped stromal cells secrete a soluble factor that could be soluble RANKL, which supports osteoclast-like cell formation in GCT [36]. RANKL was also shown to be expressed in chondroblastoma, where its involvement in osteoclastic giant cell recruitment had been suggested [37].

Altogether, data from the literature highlights the RANKL/OPG ratio's important role in malignant bone diseases. Normal stromal cells maintain the stable RANKL/OPG ratio that is required for normal bone remodelling. Stromal cells derived from GCT overexpress RANKL, which results in an increased RANKL/OPG ratio, which in turn results in an excessive development of large multinucleated osteoclasts. Myeloma and some forms of

breast carcinoma produce PTH-rP which induces RANKL and inhibits OPG production, thus resulting in an increased RANKL-to-OPG ratio that favours osteolysis and humoral hypercalcaemia of malignancy. By contrast, decreased or non modified RANKL and increased OPG production in prostate carcinoma results in a reduced RANKL/OPG ratio and may favour an osteoblastic tumour growth pattern.

II. The vicious cycle

Histological analysis of osteolytic bone metastases indicates that the bone destruction is mediated by the osteoclasts rather than directly by tumour cells. The interaction between tumour cells, tumour-derived humoral factors and the bone marrow microenvironment are crucial for the initiation and promotion of skeletal malignancies. These observations suggest a vicious cycle driving the formation of osteolytic bone tumours [38,39] (Figure 2) : tumour cells secrete soluble factors in bone (such as hormones, cytokines and growth factors), among them PTH-rP, which stimulates osteoclastic bone resorption through indirect RANKL production by osteoblastic stromal cells [40-45]. However, other data showed that tumour cells can produce themselves RANKL, acting directly on osteoclast differentiation and activation, as reported in multiple myeloma [14], prostate cancer [46], carcinoma cell lines [47] or human neuroblastoma [48]. RANKL expressed by stromal cells or directly by cancer cells can then bind to its cognate receptor RANK at the surface of osteoclast precursors and, in the presence of M-CSF, enhances the differentiation and fusion of these cells to produce functional multinucleated osteoclasts. Osteoclastic resorption in turn releases growth factors from the bone matrix, that can activate the tumour cells. In particular, Transforming Growth Factor-beta (TGF-β) is abundant in bone matrix and released as a consequence of osteoclastic bone resorption [49]. In addition to its rich stores of TGF-β, bone contains other growth regulatory factors that may act as tumour growth factors, including BMP (Bone Morphogenetic Proteins), heparin-binding fibroblast growth factors, and insulin-like growth factor I [50]. In this model, RANKL has been described as the final effector of osteoclastogenesis. The consequences of this vicious cycle are an increased tumour cell proliferation paralleled with an imbalance of bone formation/bone resorption ratio in favour of bone destruction. An experimental model of rat transplantable osteosarcoma [51] allows extending the “seed and soil” hypothesis to primary bone tumours, as development of local osteosarcoma tissue together with enhanced bone cortical destruction adjacent to the tumour can be observed in this model (Figure 2).

III. New therapeutic strategies based on OPG or RANK-Fc use

Osteolysis and tumour cell accumulation can be interrupted by inhibiting any of the vicious cycle's member, including specifically neutralizing antibodies to PTH-rP and more effective osteoclast inhibitors. Advances in osteoclast biology and pathophysiology have helped towards defining putative therapeutic targets to attack tumour-induced osteolysis. Potential therapeutic agents are being studied, including RANKL antagonists (Table 2). Based on the studies of Simonet *et al.* and Yasuda *et al.,* OPG has unambiguously been confirmed to act as a potent anti-resorptive molecule *in vivo* [7,52]. Therefore, as the majority of malignant bone diseases are characterized by enhanced bone resorption due to increased osteoclastic number and/or activity, it has been hypothesized that OPG also would be beneficial for treating animals with experimentally induced malignant bone diseases (Table 2). OPG was first shown to exhibit hypocalcaemic effects in normal mice and in hypercalcaemic nude mice carrying tumors associated with humoral hypercalcemia of malignancy [53]. OPG was then studied for its activity in a syngeneic tumour model of humoral hypercalcaemia of malignancy. It was shown to block tumour-induced increase in bone resorption and hypercalcaemia and to reduce osteoclast activity. However, it had no effect on tumour size, tumour-induced cachexia or PTH-rP levels [54].

. *Therapeutic applications of OPG*

In myeloma bone disease, OPG-Fc prevents the development of osteolytic bone lesions in 5T2 and 5T33 multiple myeloma bearing animals [14,56,57]. These changes are associated with a preservation of the cancellous bone loss induced by myeloma cells and an inhibition of osteoclast formation. In an animal model of prostate cancer (CaP), CaP cells were injected both intratibially and subcutaneously in the same mice, followed by administration of OPG [46]. OPG completely prevented the establishment of mixed osteolytic/osteoblastic tibial tumours, but it had no effect on subcutaneous tumour growth. The same conclusions were reported by Yonou *et al.,* indicating that human recombinant OPG decreased the CaP prostate burden selectively in bone, suppressed the progression of established tumour lesions and prevented the development of new lesions [58]. Honore *et al.* showed that OPG blocked bone cancer-induced skeletal destruction, skeletal pain and pain-related neurochemical reorganization of the spinal cord [59]. One year later, the same team demonstrated that administration of OPG halted further bone destruction, reduced ongoing and movement-evoked pain and reversed several aspects of the neurochemical reorganization of the spinal cord in a mouse model of osteolytic 2472 sarcoma cells injected into the intramedullary space

of the femur [60]. In the case of GCT, which represents a paradigm for the direct stimulation of osteoclast formation and activity by tumour stromal cells, *in vitro* OPG treatment potently and dose-dependently inhibited resorption of bone slices by GCT and could also inhibit the formation of multinucleated osteoclasts from precursors within the GCT [61]. To date, a single study has been reported in patients with osteolytic bone tumours : a randomised, double-blind, double-dummy, active-controlled, single dose, dose escalation study was conducted to determine the safety and effect on bone resorption of recombinant OPG in patients with multiple myeloma or breast carcinoma with radiologically confirmed lytic bone lesions [62]. The results showed that a single dose of recombinant OPG suppressed bone resorption as indicated by a rapid, sustained and profound decrease of urinary NTX/creatinine in multiple myeloma and breast cancer patients, and that recombinant OPG was well tolerated.

. *Therapeutic applications of RANK-Fc*

As reported previously in the case of prostate cancer and multiple myeloma [30,33] but also in Jurkat cells [63], OPG prevents TRAIL-mediated apoptosis of tumour cells. It could be therefore hypothesized that the clinical use of OPG may prevent TRAIL-mediated apoptosis of tumour cells. Thus, methods for blocking RANKL activity, other than OPG, may be important. For this purpose, soluble RANK-Fc, was used in a model of prostate cancer and was shown to diminish tumour-induced osteoblastic lesions, decreased serum prostate-specific antigen levels and tumour volume in the bone [64]. In myeloma, a recent study from Sordillo and Pearse reported that administration of RANK-Fc caused a marked reduction in tumour burden and serum paraprotein in the severe combined immunodeficiency (SCID)-hu-MM mouse model of human MM [65]. This was associated to limited bone destruction, restoration of OPG and reduction in RANK-L expression in the xenograft. In another recent study, Oyajobi and Mundy tested the effectiveness of antagonists of RANKL and MIP-1alpha bioactivities (RANK-Fc and neutralizing monoclonal anti-MIP-1alpha antibody) on osteolysis and tumour burden in a mouse model in which murine myeloma 5TGM1 cells are injected intravenously into syngeneic mice [66]. The results demonstrated that RANK-Fc and anti-MIP-1alpha antibody inhibited the development and progression of osteolytic lesions and significantly reduced tumour load assessed by serum monoclonal paraprotein titers. In a model of rat transplantable osteosarcoma, we developed new therapeutic approaches of primary bone tumours, and among them RANK-Fc delivery by gene therapy. The animals treated with the RANK-Fc construct exhibit higher bone apposition at the metaphysis of long

bones as shown by radiological analyses (Figure 3c), than controls treated with empty adenovirus (Figure 3b; Figure 3a : tumour controls). In this case, RANK-Fc inhibits the tumour induced-osteolysis but is not sufficient to decrease the tumour burden. In a therapeutic approach, RANK-Fc may be associated with anti-tumour drugs to stop both the tumour proliferation and the tumour-associated osteolysis. Overall, these studies demonstrate the effectiveness of RANK-Fc in inhibiting bone resorption in different models of malignant osteolytic pathologies and the upside of using RANK-Fc, which cannot interfere with TRAIL-mediated cancer cell apoptosis. Another approach lies in using novel OPG-like peptidomimetics that restore bone loss *in vivo* by facilitating a defective RANKL-RANK receptor complex, thus modulating RANK-RANKL signalling pathways and altering the biological functions of RANKL-RANK receptor complex [67]. Therefore, these OPG derived small molecules can be used to develop more useful therapeutic agents in bone diseases.

Conclusion

Skeletal complications of malignancies are catastrophic clinical events. Understanding the molecular mechanisms responsible for osteoclast activation in osteolytic primary and secondary bone tumours should lead to development of novel therapeutic approaches for these highly morbid and potentially fatal pathologies. Several factors, including IL-1, IL-6, PTH-rP, RANK Ligand and MIP-1alpha have been involved in mediating the enhanced osteoclast formation and bone destruction in patients with neoplasia. The discovery and characterization of the RANKL/RANK/OPG system has implicated RANKL and OPG as important and final mediators of deregulated bone resorption, a characteristic feature of osteolytic bone metastases and humoral hypercalcemia of malignancy. Enhanced RANKL expression plays an important role in bone destruction in patients with myeloma. RANKL is either produced directly by tumour cells or its production (by stromal bone cells or T-lymphocytes) is induced indirectly by tumour cells through secretion of PTH-rP and other cytokines. By contrast, production of the endogenous counteracting decoy receptor OPG is either inhibited or inappropriately low to compensate for the increase in RANKL.

In animal models, OPG or soluble RANK are able to control humoral hypercalcaemia of malignancy effectively, as well as the establishment and progression of osteolytic metastases caused by various malignant tumours, and to prevent cancer-induced skeletal pain and bone loss associated with immobilization. A first study assessing the beneficial effects of OPG in human multiple myeloma has been performed and reported, showing that the *in vitro*

effects of OPG can be extrapolated to the *in vivo* setting in MM patients. Beyond OPG, RANK-Fc exhibits promising therapeutic effects, as shown in animal models of prostate cancer and multiple myeloma. If the animal data translates into a similar clinical benefit in humans, RANK-Fc together with OPG may become novel potent strategies to treat patients with established or imminent malignant bone diseases in whom standard therapeutic regimens have failed.

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Legends of the figures :

Figure 1 : Osteolysis associated with primary bone tumours

Osteosarcoma (a : human, b: rat) : intense remodelling activity (arrows : osteoclasts) on tumour tissue contact (star); c. GCT: multinucleated osteoclast-like giant cells (arrows) and mononucleated cells (mnc). Original magnification, X 40.

Figure 2 : Schematic representation of the tumour cell proliferation and osteolysis vicious cycle.

Tumour cells may release soluble mediators such as hormones, cytokines, growth factors that act on osteoblastic stromal cells. The stromal cells produce RANKL, which binds to its cognate receptor RANK expressed on osteoclast precursors, enhancing the formation of active osteoclasts that carry out bone resorption. Occasionally, tumour cells have also been reported to directly release a soluble form of RANKL. Active osteoclasts then release growth factors, cytokines or bone matrix components stored in the bone matrix that in turn activate tumour cell proliferation. The vicious cycle thus induces both tumour cell proliferation and bone resorption.

Figure 3 : Radiographs of osteosarcoma-bearing Sprague-Dawley rats, treated or not with adeno (Ad)-RANK-Ig.

Osteosarcoma tumours were implanted contiguous to the tibia of male Sprague-Dawley rats on day 0. Eighteen days after, the rats were injected in an intra-portal situation with empty adenovirus (b) or with Ad-RANK-Ig (c), and compared to controls (a). Note the intense bone apposition at the metaphysis of long bones in Ad-RANK-Ig treated rats (c).

Table 1 : Malignant metabolic bone diseases with involvement of the RANKL/OPG system

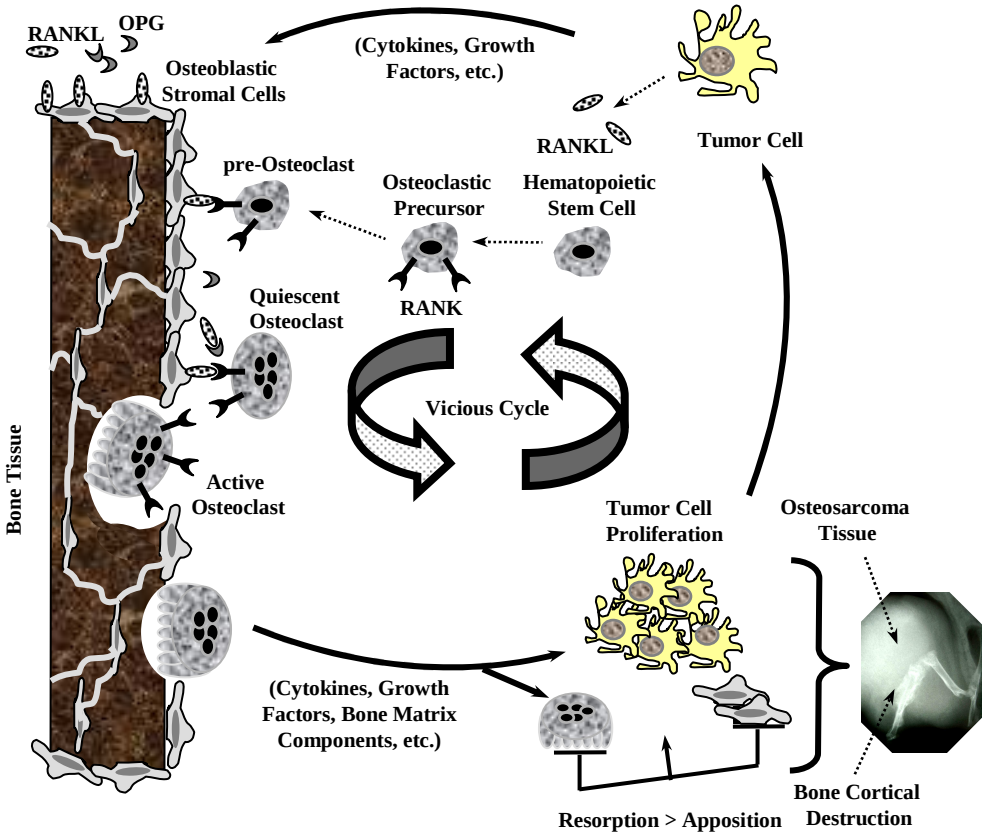
Pathology	references
. Expression of RANKL by bone tumors	[9]
. RANKL/OPG ratio is increased in severe osteolysis	[10]
. RANKL/OPG expression in multiple myeloma	[12,13-15]
. Disturbance in the OPG/RANKL balance in the bone marrow environment of myeloma cells	[11,16,17]
. OPG in serum of patients with multiple myeloma	[18,19]
. OPG may be a survival factor for human myeloma cells	[33]
. RANKL/OPG ratio as a novel prognostic index in multiple myeloma	[21]
. RANKL in adult T-cell leukaemia cells	[22]
. RANKL expression in skeletal metastases	[23]
. OPG and RANKL expression in the management of patients with skeletal metastases	[24]
. RANKL expression by stromal cells in breast cancer	[25]
. RANKL/OPG in breast cancer metastases	[26]
. RANKL expressed by prostate cancer skeletal metastases	[27,46]
. RANKL expressed by carcinoma cell lines	[47]
. RANKL expressed in human neuroblastoma	[48]
. OPG in human prostate cancer	[30]
. RANKL in cancer-associated osteolytic lesions	[25]
. RANKL/OPG in Giant Cell Tumor	[34-36]
. RANKL expression in chondroblastoma	[37]
. RANKL in hypercalcemia	[47]

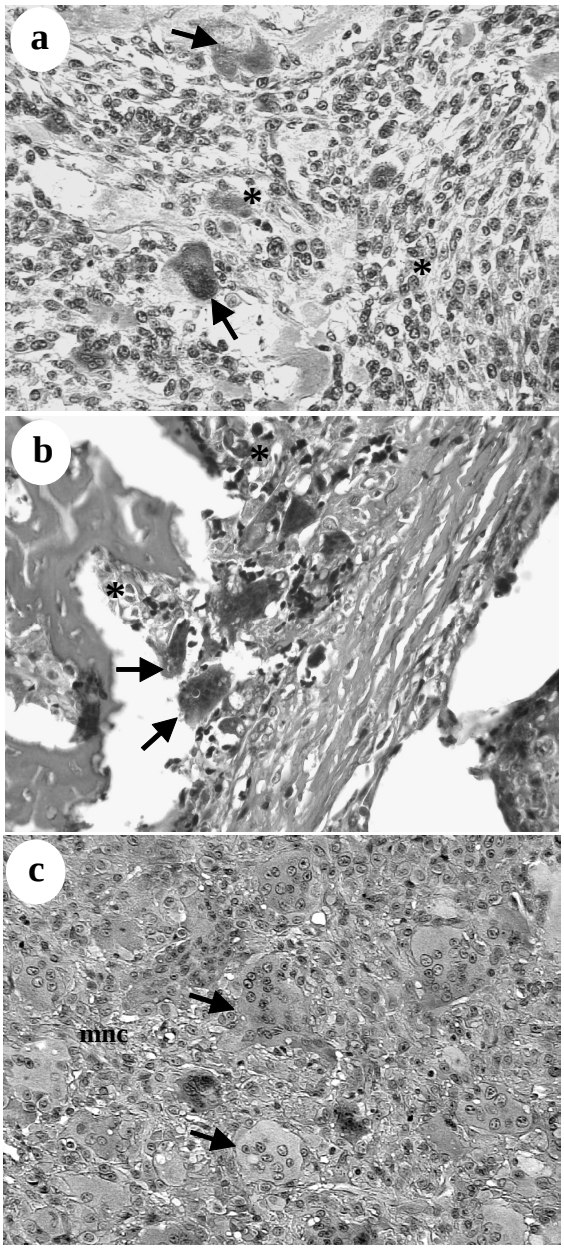
Table 2 : Therapeutic strategies based on OPG/RANK/RANKL triad

Pathology	references
. OPG prevents and reverses hypercalcemia malignancy	[53,54]
. Inhibition of osteoclastogenesis and tumor growth in osteopetrotic mice by OPG	[55]
. OPG inhibits osteolysis in multiple myeloma and increases survival in a murine model	[56,57]
. OPG inhibits human prostate cancer burden in bone in an immunodeficient mice model	[58]
. Serum OPG as a marker of bone metastatic spread in prostate cancer	[31]
. OPG inhibits osteoclastogenesis and prevents prostate tumor growth	[46]
. Single dose OPG in patients with bone metastases from multiple myeloma	[62]
. Inhibition of cancer-induced skeletal destruction by OPG	[59]
. OPG diminishes advanced bone cancer pain	[60]
. RANK-Fc : a therapeutic antagonist for RANKL in myeloma	[65,66]
. sRANK-Fc diminishes tumor volume in prostate cancer	[64]

Table 1 : Malignant metabolic bone diseases with involvement of the RANKL/OPG system and therapeutic strategies based on OPG/RANK/RANKL triad

Pathology	References
RANKL and RANK expression in multiple myeloma	Roux et al, 2002 ; Farrugia et al 2003
Disturbance in the OPG/RANKL balance in the bone marrow environment of myeloma cells	Giuliani et al, 2001 ; Pearse et al, 2001 ; Giuliani et al, 2002 ; Yaccoby et al, 2002
OPG may be a survival factor for human myeloma cells	Shipman and Croucher, 2003
RANK-Fc: a therapeutic antagonist for RANK-L in myeloma	Sordillo and Pearse, 2003
Single dose in a single dose in patients with bone metastaes from multiple myeloma (clinical trial) OPG inhibits oteolysis in multiple myeloma and increases survival in a murine model	Bekker et al, 2001 Croucher et al, 2001 ; Vanderkerken et al, 2003
RANKL/OPG ratio as a novel prognostic index in multiple myeloma	Terpos et al, 2003
RANKL as a prerequisite for cancer-associated osteolyic lesions	Kitazawa et al, 2002
OPG diminishes advanced bone cancer pain	Luger et al, 2001
Inhibition of cancer-induced skeletal destruction by OPG	Honore et al, 2000
RANKL expression in skeletal metastases	Huang et al, 2002
Expression of RANKL by bone tumors	Good et al, 2002
RANKL/OPG ratio is increased in severe osteolysis	Grimaud et al, in press
OPG and RANKL expression in the management of patients with skeletal metastases	Demers et al, 2003
Inhibition of osteoclastogenesis and tumor growth in osteopetrotic mice by OPG	Clohishy et al, 2000
OPG inihibits human prostate cancer burden in bone in an immunodeficient mice model	Yonou et al, 2003
Serum OPG as a marker of bone metastatic spread in prostate cancer	Jung et al, 2001
OPG inhibits osteoclastogenesis and prevent prostate tumor growth	Zhang et al, 2001
OPG prevents ans reverses hypercalcemia malignancy	Akatsu et al, 1998; Capparelli et al, 2000


RANKL
OPG
Osteoblastic
Stromal Cells
(Cytokines, Growth
Factors, etc.)
RANKL
Tumor Cell
pre-Osteoclast
Osteoclastic
Precursor
Hematopoietic
Stem Cell
RANK
Quiescent
Osteoclast
Vicious Cycle
Bone Tissue
Active
Osteoclast
Tumor Cell
Proliferation
Osteosarcoma
Tissue
(Cytokines, Growth
Factors, Bone Matrix
Components, etc.)
Resorption > Apposition
Bone Cortical
Destruction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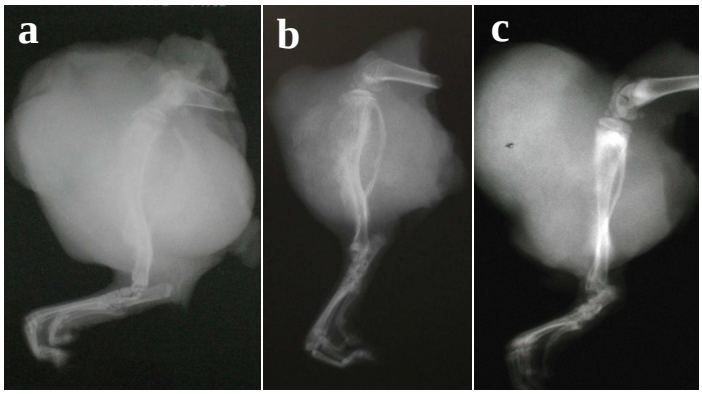
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